GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *PTERIS VITTATA* EXTRACT AND IT'S ANTIMICROBIAL ACTIVITY

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

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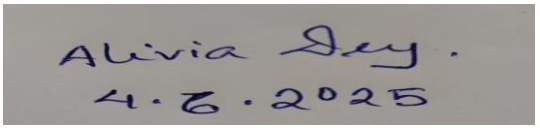

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COURSE COMPLETION CERTIFICATE

This is to certify that Alivia Dey (Registration ID: 230020440135; Enrollment ID: 2322210002044), a student of M.Sc. in Biotechnology, has successfully completed her final semester dissertation work under my guidance, Dr. Joy Sarkar, Associate Professor of Botany, at Dinabandhu Andrews College, Kolkata.

The dissertation work was carried out as a part of the academic requirement for the partial fulfillment of the degree of Master of Science in Biotechnology under the School of Health Science and Translational Research, Sister Nivedita University, Kolkata.

The research project entitled *"Green Synthesis of Silver Nanoparticles using Pteris Extract and it's Antimicrobial Activity"* was conducted during the period January 2025 to June 2025, and the student has shown sincere dedication and scientific aptitude throughout the course of the work.

I wish the student every success in future academic and professional endeavors.

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ABBREVIATIONS:

AgNo3 NPs – Silver nitrate Nanoparticles

FTIR – Fourier Transform Infrared Spectroscopy

DLS_ Dynamic Light Scattering

XRD- X- ray Diffraction

UV – Vis – Ultraviolet visible Spectroscopy

ABSTRACT:

Norio Taniguchi was the first to use the word "nanotechnology" in 1974. He first used it to explain how materials are manipulated at the nanoscale, with a particular emphasis on the production of semiconductors.

Nanotechnology has greatly impacted a variety of technologies, including mechanical, electrical, thermal, optical, and biological ones, and it has broadened scientific possibilities. In the past, techniques for producing nanoparticles included chemical vapor deposition, microchemical cleavage, and the application of extremely hazardous and reactive chemical reductants. As a result, the procedure was costly, time-consuming, and difficult to control. Large-scale nanoparticle synthesis became essential as nanoscience developed, necessitating the creation of safer, more economical, and faster synthesis methods. Out of all the alternative synthetic strategies that were studied, the biosynthesis of nanoparticles quickly became one of the main methods for bulk nano synthesis. Certain nanoparticles have been claimed to be produced by a variety of bacteria, fungi, and algae.

The purpose of this study was to determine the antibacterial activity of silver nanoparticles (Ag-NPs) by using a low-cost green synthesis technique to formulate Ag-NPs using plant (*Pteris vittata)* leaves. Silver nanoparticles were characterized by techniques such as X-ray diffraction analysis, dynamic light scattering, and UV-visible spectroscopy ,and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The generated silver nanoparticles had a spherical shape and ranged in size from 4 to 15 nm. The crystal structure of the nanoparticles was a face-centered cubic. The selected method for the synthesis of silver nanoparticles is suitable for large-scale production.

The as-prepared silver nanoparticles demonstrated exceptional antibacterial activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria, and they would make a good candidate for several kinds of pharmaceutical and environmental applications.

KEYWORDS: green synthesis, antimicrobial activity, *Pteris vittata*, silver nanoparticles

1. INTRODUCTION:

DEFINITION

Nanotechnology is the study of modifying matter at the near-atomic scale to produce new electronics, materials, and structures that thrive at the nanoscale. This technology is essential to the growth and development of many scientific disciplines, including manufacturing, energy, materials science, engineering, and medicine. (Rai et al., 2008)

In the metric system, a nanometer is a unit of length. The English word "nano" comes from the Greek word "Nanos," which means "dwarf" or "very small." Technically speaking, a centimeter is equivalent to one hundredth of a meter, and a nanometer (nm) is one billionth of a meter. As a result, all nanoscale structures typically have a length or dimension range of 1 to 100 nanometers .

HISTROY OF NANOTECHNOLOGY

The history of nanotechnology traces back to ancient times when civilizations unknowingly utilized nanomaterials, such as in the Roman Lycurgus Cup and medieval stained glass, which contained gold and silver nanoparticles that gave them unique optical properties. However, physicist Richard Feynman's 1959 lecture, "There's Plenty of Room at the Bottom," imagined the prospect of controlling individual atoms and molecules, laying the conceptual groundwork for nanotechnology. Japanese physicist Norio Taniguchi later came up with the term "nanotechnology" in 1974. (Nadeem joudeh & Drink Linke , 2022) Significant advancements occurred in the 1980s with the invention of the Scanning Tunneling Microscope (STM) in 1981, allowing scientists to visualize atoms for the first time, and the discovery of fullerenes (C60) in 1985. In 1991, carbon nanotubes were discovered, opening new avenues in material science. The U.S. government launched the National Nanotechnology Initiative in 2000 to promote research and commercialization. Since then, nanotechnology has found applications in medicine, electronics, energy, and materials, with innovations such as graphene and nano-drug delivery systems leading modern research. Today, nanotechnology continues to evolve, promising revolutionary advancements across science and industry.(Nadeem joudeh & Drink Linke, 2022)

Why nanomaterials are different

Nanomaterials are different from their bulk counterparts primarily due to their extremely small size, typically less than 100 nanometers, which gives rise to unique physical, chemical, and biological properties. One of the most significant differences is their large surface area to volume ratio, which increases their reactivity and enhances interactions with other substances. Additionally, quantum

effects become prominent at the nanoscale, altering electrical, optical, and magnetic behaviors in ways not seen in larger materials. These effects can lead to novel properties such as size-dependent fluorescence or enhanced strength and flexibility. Nanomaterials may also exhibit unusual thermal and electrical conductivity, as well as increased chemical reactivity, due to the high proportion of surface atoms. These distinct characteristics make nanomaterials highly valuable in various applications, including medicine, electronics, catalysis, and environmental remediation. (Vijay Kanad, 2023)

Classification of nanomaterials

Generally there are three types of Nanoparticles. Organic nanoparticles: this types of nano particles is made by organic source like proteins, carbohydrates, lipids. Example- Liposomes, micelles.

Inorganic nanoparticles: made by metals and metal oxide. Example- silver, zinc, cu nanoparticles.

Carbon-Based Nanoparticles: These are made from carbon-based materials like nanotubes, graphene.

Table 1.1 Some types of nanoparticles and their common applications:

Type of Nanoparticle	Composition	Size Range (nm)	Common Applications
Metallic Nanoparticles	Gold (Au), Silver (Ag), Platinum (Pt), etc.	1–100	Drug delivery, biosensing, imaging, catalysis
Metal Oxide Nanoparticles	TiO_2, ZnO, Fe_3O_4, CuO	10–100	Sunscreens, pigments, antimicrobial coatings, sensors
Quantum Dots	CdSe, CdTe, PbS, InP	2–10	Bioimaging, LED displays, photovoltaics
Carbon-Based Nanoparticles	Fullerenes, Carbon Nanotubes, Graphene	1–100	Electronics, drug delivery, energy storage
Polymeric Nanoparticles	Biodegradable polymers (PLGA, PEG, etc.)	10–1000	Controlled drug release, vaccine delivery
Lipid-Based Nanoparticles	Liposomes, Solid Lipid Nanoparticles (SLNs)	50–1000	mRNA delivery (e.g., COVID-19 vaccines), cancer therapy

Ceramic Nanoparticles	Silica, alumina, calcium phosphate	10–100	Bone regeneration, dental materials, catalysts
Magnetic Nanoparticles	Iron oxide (Fe_3O_4, γ-Fe_2O_3)	5–100	MRI contrast agents, hyperthermia treatment, separation
Dendrimers	Branched synthetic polymers	1–10	Drug delivery, gene therapy, nanomedicine
Composite Nanoparticles	Combination of different materials	Varies	Multifunctional delivery, advanced sensors

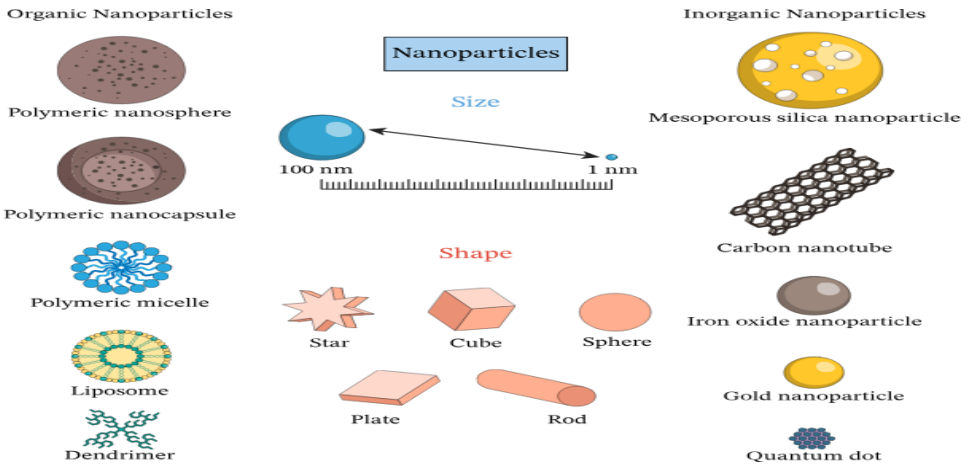


FIG 1.1 Types of Nanoparticles

HISTROY OF SILVER NANOPARTICLES

Due to global issues connected to environmental concerns, "green" or ecologically friendly chemical and chemical technology processes are becoming more and more popular. (Rafique et al., 2017)Silver is the one of the most leverage nano- material. Five hundred tons of silver nanoparticles are produced year, and this number is expected to rise over the next few years, making silver one of the most industrialized nanomaterials. In the form of metallic silver, silver nitrate (AgNO3), and silver sulfadiazine, silver has been used since 1000 B.C. to treat burns, wounds, and a number of bacterial illnesses. (International journal of Green Nanotechnology, 2012)

However, with the discovery of penicillin in the 1940s, the use of silver compounds in medical systems significantly decreased. However, the field of nanotechnology is currently bringing about a new era of science by producing materials with unique chemical, physical, thermal, and optical properties at the near-atomic size. Ag-NP, a metallic silver derivative with strong antibacterial qualities, consequently made a spectacular resurgence. Ag-NP has exceptional activity against a variety of viruses, fungi, and bacteria. According to research,

after being exposed to bacteria, the nano-silver sticks to the cell membrane, enters the cell, targets the respiratory chain, and ultimately causes cell death. Additionally, inside cells, these particles generate silver ions that deactivate thiol- group proteins and stop bacteria from multiplying. The new techniques for using pure silver against a range of antibiotic-resistant pathogens have led to its use in many commercial products, such as toothpaste, soap, socks, and topical ointments. (Asian Journal of Pharmaceutical and clinical Research , June 2019)

Due to their antibacterial qualities, silver nanoparticles are used in water filters and purification systems to eliminate bacteria. By inducing apoptosis in cancer cells, it may also aid in cancer treatment. Ag-NP can be used as targeted drug delivery vehicles, enhancing the efficacy of medications and reducing side effects. Ag-NP is used in active packaging materials to prolong shelf life by preventing microbial growth. applied to textiles to give them self-cleaning, antibacterial properties.

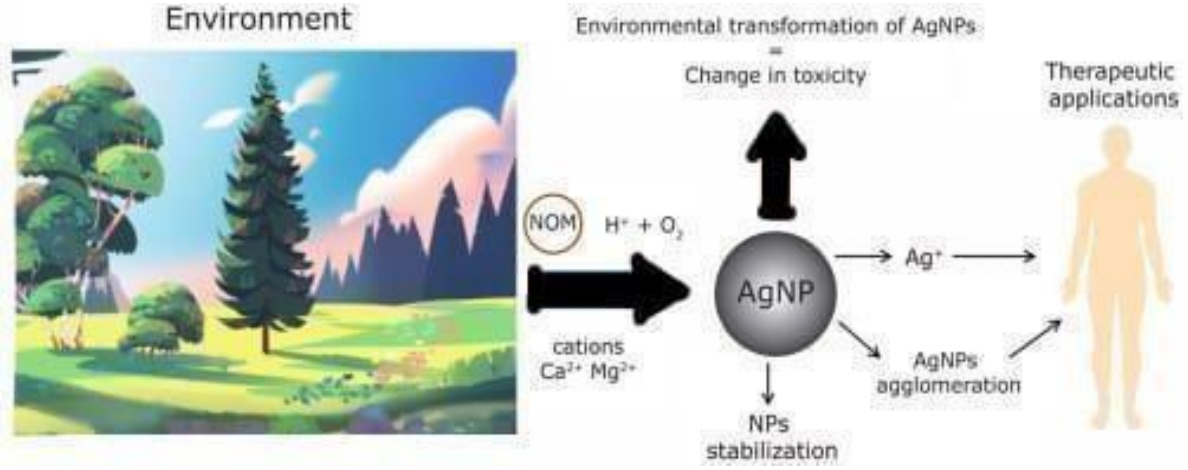


Fig1. 2: Schematic diagram of application of silver nanoparticles on environment

Table 1.2 **Nanoparticles synthesis by plant extract:**

Source (Example Plant)	**Type of Nanoparticle Synthesized**	**Role in Synthesis**	**Common Applications**	**Bioactive Compound**
Neem (*Azadirachta indica*)	Zinc oxide (ZnO), Silver	Reducing & stabilizing agent	Antimicrobial	**Flavonoids**
Eucalyptus	Gold, Silver	Reducing agent	Antiviral, anti-inflammatory	**Terpenoids**
Clove (*Syzygium aromaticum*)	Silver, Copper	Reducing agent	Antimicrobial	**Tannins**

Papaya (*Carica papaya*)latex	Gold, Silver	Capping & reducing agent	Cancer targeting	**Proteins/Enzymes**
Aloe vera, Banana peel	Gold, Silver	Reducing agent	Wound healing	**Carbohydrates**
Lemongrass, Tea tree	Silver, Zinc oxide	Reducing & stabilizing agent	Antifungal, anti-inflammatory	**Essential Oils**
Green tea (*Camellia sinensis*)	Silver (AgNPs), Gold (AuNPs)	Reducing & capping agent	Antibacterial, drug delivery	**Polyphenols**
Tulsi (*Ocimum sanctum*)	Gold, Silver	Reducing agent	Cancer treatment	**Alkaloids**

SILVER NANOPARTICLES SYNTHESIS BY PTERIS EXTRACT

In this instance, I'm going with the environmentally friendly production of silver nanoparticles using pteris extract. because there are several practical, scientific, and environmental reasons why it is a beneficial strategy. Ag ions are converted to Ag nanoparticles by bioactive phytochemicals such as flavonoids, terpenoids, and polyphenols, which are prevalent in Pteris species. To stabilize the nanoparticles and prevent them from aggregating, cap them. Pteris is an inexpensive, easily obtainable plant. enhanced antibacterial, antioxidant, or anticancer qualities due to the compound's and silver's synergistic relationship with plants. Using Pteris may be a new tactic in comparison to more commonly used plants. Lastly, it might

be published due to its distinctive qualities. (DOI: 10.5772/intechopen.1002203)

Table 1.3 Activated Biological Compounds in *Pteris* Extract:

Compound	Role in AgNPs Synthesis	Contribution to Antimicrobial Activity
Flavonoids	Function as both **reducing and capping agents**	Interact with bacterial cell walls, leading to lysis
Tannins	Provide **reduction and stabilization**	Bind to microbial proteins and enzymes and then destroy the cell.
Phenolic compounds	Act as strong **reducing agents**, converting $Ag^{+} \rightarrow Ag^{0}$	Disrupt microbial membranes through oxidative stress

Pteris, a genus of ferns, is considered effective for the synthesis of silver nanoparticles due to its rich phytochemical composition, including flavonoids, phenolics, terpenoids, and alkaloids. These natural compounds act as both reducing and stabilizing agents in the green synthesis process, facilitating the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) without the need for harmful chemicals. Additionally, Pteris species are widely available, easy to cultivate, and environmentally friendly, making them a sustainable choice for nanoparticle production. The plant extract's strong antioxidant activity further enhances its ability to produce stable and well-dispersed nanoparticles, making Pteris a valuable biological resource in nanotechnology applications.

2. Review of Literature:

Silver Nanoparticles synthesis by many types of plants:

Green synthesis is the process of creating silver nanoparticles (AgNPs) using a wide range of plant extracts. This economical and environmentally friendly process converts silver ions (Ag^{+}) into metallic silver (Ag^{0}) nanoparticles by using phytochemicals found in plants. The reduction and stabilization processes depend heavily on plant metabolites such as flavonoids, alkaloids, terpenoids, polyphenols, tannins, and proteins.

Common Plants Used for Silver Nanoparticle Synthesis:

For examples,

Ocimum sanctum **(Tulsi)** – Rich in antioxidants and phenolic compounds.

Azadirachta indica **(Neem)** – high antimicrobial activity.

Aloe vera – gel contains polysaccharides .

Wheat (*Triticum aestivum*) – grass juice used as a reducing agent.

Rice (*Oryza sativa*) – husk extracts and straw are also used.

Green Synthesis of AgNPs using Pteris Extract:

Utilizing the natural reducing and stabilizing compounds found in the plant, the green synthesis of silver nanoparticles using Pteris extract is an economical and

environmentally beneficial process. Fresh Pteris leaves are washed in distilled water to create a phytochemical-rich extract that contains tannins, phenols, and flavonoids. The phytochemicals in this extract then convert silver ions (Ag^{+}) to metallic silver nanoparticles (Ag^{0}), as shown by a color shift from pale yellow to dark brown, when combined with a silver nitrate ($AgNO_3$) solution. Characterization of the produced nanoparticles can be done with methods such as FTIR, SEM, XRD, and UV-Vis spectroscopy. In addition to avoiding dangerous chemicals, this green synthesis provides a sustainable method of creating silver nanoparticles that may find use in environmental cleanup, antimicrobial therapy, and medicine.

Key Phytochemicals Found in *Pteris* spp.

Numerous bioactive phytochemicals found in the genus Pteris, which includes ferns, are known to have therapeutic benefits and to aid in the synthesis of nanoparticles. In green synthesis, these substances serve as organic stabilizing and reducing agents.

Flavonoids

Flavonoids is an antioxidants and reducing agents. It helps in the reduction of silver ions(Ag+) to nanoparticles.

Phenolic Compounds

Phenolic Compounds exhibit strong reducing and antimicrobial activity. It plays a role in stabilization of nanoparticles.

Tannins

Tannins is a Polyphenolic compounds with astringent properties which Contribute to both reduction and capping of nanoparticles.

Terpenoids

Terpenoids is an Aromatic compounds that may help in capping and stabilizing nanoparticles.

Saponins

Saponins is a Natural surfactants that may help in nanoparticle dispersion.

Alkaloids

Some species of Pteris plant may contain alkaloids with medicinal properties.

Steroids

Steroids is a phytochemical component that Contribute to structural and bioactive functions.

Mechanism of synthesis:

The synthesis of AgNPs using *Pteris* plant extract involves a green, bioreduction process facilitated by some phytochemicals present in the plant.

Flavonoids are found in *Pteris* plant . It is a polyphenolic compounds. It is contained by multiple **hydroxyl (–OH)** groups. These groups helps to play key role in good **electron donors**, which is critical in reducing Ag^{+} ions to Ag^{0}.

Flavonoid-OH +Ag+→Flavonoid-Ag+ complex

Flavonoid-OH → Flavonoid=O+2H++2e−

Ag++e− →Ag0

Flavonoid interact to bacterial cell wall and leading to lysis.

Tannins is another bioactive compound which is found in *Pteris* plant.

It Provide reduction and stabilization.

It helps to bind with microbial proteins and enzymes and then destroy the microbial cell.

Phenolic compounds , phytochemical compound that act as strong reducing agents and converting $Ag^{+} \rightarrow Ag^{0}$

It helps to disrupt microbial membranes through oxidative stress and kill the microbes.

Terpenoids is another essential agent that may assist in reduction and stabilization.

Pteris plant's advantages in green synthesis:

Pteris ferns have shown potential in green synthesis, particularly in the environmentally safe synthesis of nanoparticles. By using biological entities like fungi, bacteria, and plants to produce nanoparticles, green synthesis avoids harsh conditions and dangerous chemicals.

- **Eco-friendly and sustainable:**

Because fewer hazardous chemicals are needed for synthesis when *Pteris* plants are used, the process is non-toxic and biodegradable, making it perfect for applications involving green chemistry**.**

- **Cost-effective and easily available:**
- *Pteris* is an easily cultivated fern, making it a low-cost alternative to expensive chemical reagents. Also it is a common fern.
- **Phytochemical-rich extracts**:
- Phenols, flavonoids, tannins, and terpenoids, which are rich in *Pteris* species, serve as organic capping and reducing agents during the synthesis of nanoparticles.
- Ag^{+} and Au^{*+} are examples of metal ions that these chemicals help in reducing to their metallic nanoparticle forms.
- **Biocompatibility**:
- Nanoparticles synthesized using *Pteris* plant extracts is a very cost effective and tend to be **biocompatible**, making them suitable for medical and biological applications, such as drug delivery and antimicrobial activity, anticancer treatment.
- **Rapid synthesis**:
- The bioactive agents in *Pteris* can advance nanoparticle formation, enabling **quick and efficient synthesis** at room temperature without the need for high energy inputs.

Application of *Pteris* mediated silver nanoparticles:

Pteris-mediated silver nanoparticles (AgNPs) are an aspect of green nanotechnology, which uses plant extracts as stabilizing and reducing agents during the manufacturing of nanoparticles. Pteris species are a type of fern. Because of their distinct physicochemical and biological characteristics, these nanoparticles have a wide range of uses.

1. **Antibacterial and Antimicrobial Activity**
   - **Primary use**: AgNps have strong antibacterial and antimicrobial activity against both Gram positive and Gram negative bacteria.

- **Mechanism**: They binds with microbial cell membrane and disrupt the cell membranes, generate reactive oxygen species (ROS), and interfere with cellular processes.
- **Application**: Generally use in Coatings for medical devices, antimicrobial packaging, wound dressings, etc.

2. **Antifungal and Antiviral Properties**

- AgNPs synthesized using *Pteris* can also inhibit the growth of various fungi and some viruses.

- Due to the excellent antifungal activity, It use in agricultural field (crop protection) and healthcare (surface disinfectants, sprays).

3. **Antioxidant Activity**

- Pteris-based AgNPs may possess free radical scavenging abilities due to many types of phytochemicals in the plant extract. Such as Tannins, Flavonoids etc.

- **Application**: Due to the antioxidant activity, it use in Pharmaceutical and cosmetic products to reduce oxidative stress.

4. **Catalytic Applications**

- They have the ability to catalyze chemical reactions, such as the reduction of contaminants or the breakdown of colors.

- **Use case**: Environmental remediation and industrial processes.

5. **Anti-cancer Potential**

- They have also cytotoxic effects of AgNPs on cancer cells.

- The phytochemicals from *Pteris* may enhance this effect.

- **Application**: Research in cancer therapy.

6. Biosensing and Diagnostic Applications

- AgNPs exhibit surface plasmon resonance (SPR), making them useful in biosensors.
- Can be used for detecting biomolecules contaminants.

6. **Eco-Friendly Alternative**

- The *Pteris*-mediated synthesis is green, non-toxic, and cost-effective.
- **Use**: Sustainable nanomaterial products.

Pteris and the Formation of Silver nanoparticles:

The size, shape, surface charge, and dispersion of silver nanoparticles (AgNPs) are among its physicochemical properties that have a major impact on their biological activity. It is produced by *Pteris* plant extract.

They usually have the following characteristics:

They range in size from 10 to 80 nanometers and are spherical or almost spherical in shape. Depending on the synthesis conditions, they exhibit distributions that are either monodispersed or somewhat agglomerated.
A number of characterization techniques have been used to verify the creation of these nanoparticles and to pinpoint the functional groups responsible for their stabilization and reduction, such as Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-ray diffraction (XRD), and UV-visible spectroscopy.

Furthermore, AgNPs have surface plasmon resonance (SPR), as seen by UV -Vis absorption peaks that are usually located between 350 to 800 nanometer.

3. MATERIALS AND METHODS :

Objective: The main objective of the dissertation work is ;

- Prepare nanoparticles from Pteris extract.
- Characterization of nanoparticles by UV-Visible Spectrophotometry, XRD, and FTIR.

- Identify the antimicrobial activity of nanoparticles by Agar Diffusion method and Broth Dilution Assay.

Materials :

Silver nitrate (AgNO3), Pteris Plant extract, Distilled water, NaOH, Nutrient broth (NB), Luria-Bertani broth (LB), Iodonitrotetrazolium chloride (INT dye), Alcohol.

Preparation of Pteris Leaf Extract:

Plant Collection and washing:

The Pteris leaves were collected from a botanical garden. A botanist examined the leaves to verify the species identification. The collected samples stored in a paper bag for further use.

Fig 3.1: Pteris Leaves

Wash it thoroughly with tap water, followed by distilled water to remove dust and other contaminants.

Extraction:

Chop the leaves into small pieces.

Crushing and grinding these small pieces by motar pestle with adding distilled water.

Allow to filter the mixture by using filter paper (Bloating paper) to obtain a clear extract.

Synthesis of Silver Nanoparticles:

Preparation of silver nitrate solution:

Prepare an aqueous solution of AgNo3 0.01mM concentration.

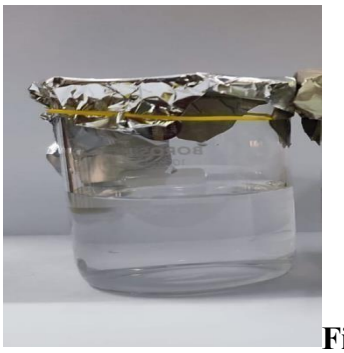


Fig 3.2: AgNO3 solution preparation

Reaction:

Mix the plant extract with the AgNo3 solution in a 2:8ratio.

Stir the mixture at room temperature and heat gently (40-70º) for 10 minutes .

Observation:

The colour change from brown to dark brown indicates the formation of silver nanoparticles due to the surface plasmon resonance.

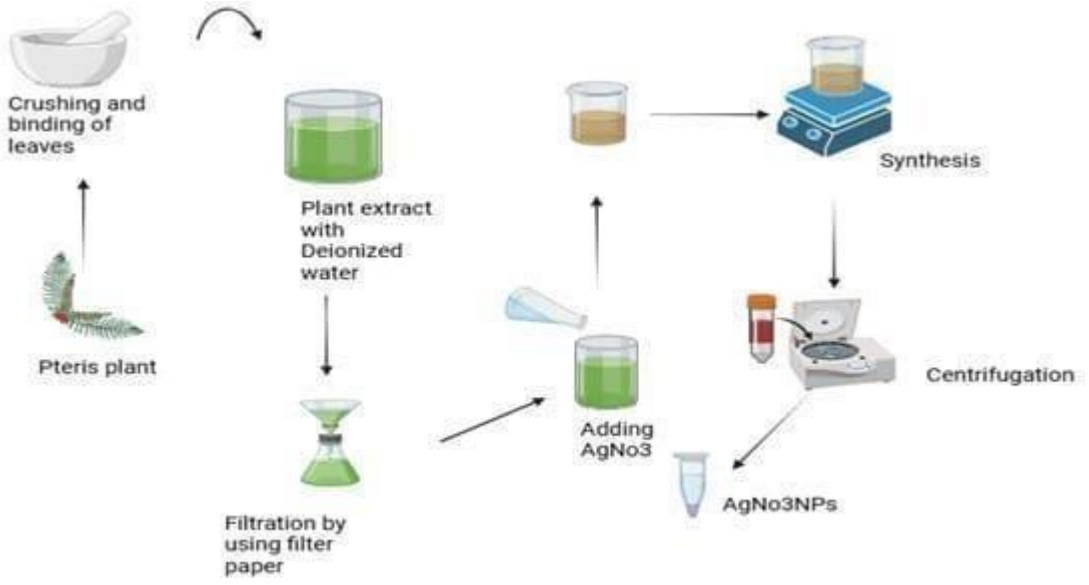
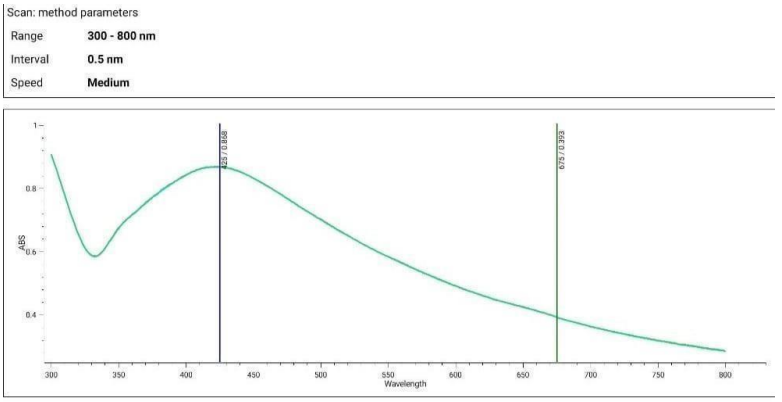


Fig 3.3 :Schematic illustration of synthesis of silver nanoparticles created with Biorender.com

Characterization :

- *UV-Vis Absorption Spectroscopy*

UV-Visible absorption spectroscopy is a fundamental technique used to confirm the formation and study the optical properties of silver nanoparticles (AgNPs). Silver nanoparticles exhibit a characteristic surface plasmon resonance (SPR) peak in the UV-Visible region, typically between 300-800 nm, depending on factors such as particle size, shape, and the surrounding medium. This SPR peak arises due to the collective oscillation of conduction band electrons on the nanoparticle surface in response to incident light. The appearance of a sharp and well-defined peak in this region indicates the successful synthesis of AgNPs. A shift in the peak position or broadening of the peak may suggest changes in particle size, aggregation state, or surface chemistry. UV-Vis spectroscopy is thus a quick and reliable method not only for confirming nanoparticle formation but also for monitoring their stability and dispersion over time.



Sample	ABS(425)	ABS(675)
AgNO3 TE nano 1	0.868	0.393
AgNO3 TE nano 2	0.868	0.393
AgNO3 TE nano 3	0.869	0.394

Fig 3.4: **UV-Vis Absorption Spectroscopy analysis of Silver nanoparticles and the wavelength between 300 to 800 nm.**

- ***Dynamic Light Scattering (DLS)***

.Dynamic Light Scattering (DLS) is a widely used technique to determine the size distribution and hydrodynamic diameter of nanoparticles in a colloidal suspension. When applied to silver nanoparticles, DLS helps characterize their dispersion stability and average particle size in solution. In DLS, a laser light is

scattered by the nanoparticles undergoing Brownian motion, and the fluctuations in the scattered light intensity are analyzed to calculate the diffusion coefficient. This data is then used to determine the particle size based on the Stokes-Einstein equation. Silver nanoparticles typically show a narrow size distribution when synthesized under controlled conditions, and DLS provides valuable insights into agglomeration, surface modification effects, and overall colloidal behavior. The technique is non-destructive, rapid, and suitable for routine quality control and research purposes in nanomaterials science.

- ***Fourier transfigure Infrared Spectroscopic (FTIR) Analysis system***

Fourier Transform Infrared (FTIR) spectroscopy is a crucial analytical technique used to characterize silver nanoparticles (AgNPs), particularly to identify the functional groups present on their surface. During the synthesis of AgNPs, various biomolecules or chemical agents act as reducing and stabilizing agents, and FTIR analysis helps confirm their involvement. The FTIR spectrum typically shows characteristic peaks corresponding to different functional groups, such as hydroxyl (–OH), carbonyl (C=O), amine (–NH), or carboxyl (–COOH), indicating the presence of proteins, phenols, or other organic molecules that cap the nanoparticles. These surface functional groups are important as they influence the stability, dispersibility, and biological interactions of AgNPs. A shift in peak position or the appearance of new peaks in the FTIR spectrum compared to the original capping agent confirms the successful synthesis and surface modification of silver nanoparticles. Therefore, FTIR plays a vital role in understanding the chemical environment around AgNPs and in verifying their successful functionalization.

The culture filtrate's preliminary vacuum-dried inorganic essence nanoparticles were combined separately with potassium platitude (KBr) at a rate of 1100 (w/w). Both components were mashed entirely with a pestle and mortar, then combined and placed inside a spherical vessel in a two-piston bullet press. For a variety of ages, bullets were created using a vacuum atmosphere with pressures exceeding 25,000 psi. The spectrophotometer holder was being filled with the transparent bullets. Because KBr is transparent to the infrared diapason, a full infrared wavelengths in the 4000 – 400 cm^{-1} range was attained using a Shimadzu 8400S FTIR spectrophotometer using a verbose reflection accessory, comprising 50 reviews per sample.

- ***X- Ray diffraction (XRD) Analysis:***

XRD analysis was used to determine and validate the crystal structure of the generated silver nanoparticles. . When subjected to XRD, silver nanoparticles typically exhibit distinct diffraction peaks corresponding to the face-centered cubic (FCC) structure of metallic silver. The most prominent peaks are usually observed at 2θ values around 38.1°, 44.3°, 64.4°, and 77.5°, which correspond to the (111), (200), (220), and (311) crystallographic planes, respectively, as indexed by the JCPDS (Joint Committee on Powder Diffraction Standards) card for silver. These peaks shows the sharpness and intensity which indicates the crystalline nature of the nanoparticles. Additionally, the average crystallite size can be calculated using the Scherrer equation based on the full width at half maximum (FWHM) of the diffraction peaks. XRD analysis not only confirms the formation of pure silver nanoparticles but also helps identify any secondary phases or impurities, making it an essential tool for structural characterization in nanomaterials research.

- **Antimicrobial property of the AgNo3 NPs:**

Agar well diffusion assay :

The agar well diffusion assay is a commonly used method to evaluate the antimicrobial activity of silver nanoparticles (AgNPs). In this technique, a sterile agar plate is inoculated with a microbial culture, typically microbes, to create a uniform lawn of growth. Wells are then punched into the agar using a sterile borer, and various concentrations of silver nanoparticles are introduced into these wells. The plate is incubated under suitable conditions to allow microbial growth. During this period, the silver nanoparticles diffuse from the wells into the surrounding agar. If the nanoparticles possess antimicrobial properties, they inhibit the growth of microorganisms in the vicinity of the wells, resulting in clear zones known as zones of inhibition. The diameter of these zones is measured to assess the effectiveness of the silver nanoparticles against the test organism. Larger zones indicate stronger antimicrobial activity. This assay provides a simple, cost-effective, and qualitative method for screening the antimicrobial potential of silver nanoparticles and comparing their efficacy against different microbial strains.

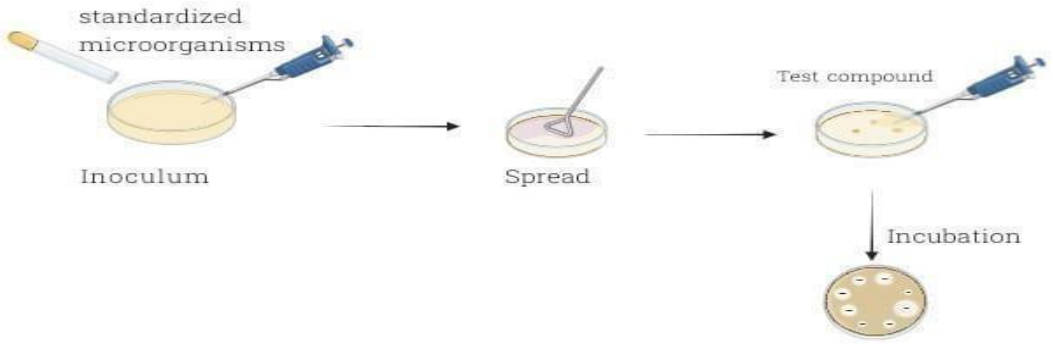


Fig3.5: Agar well diffusion assay technique

Broth Microdilutionassay:

A common technique for assessing the antimicrobial activity of materials such as silver nanoparticles (AgNPs) is the broth microdilution assay, which measures the substances' Minimum Inhibitory Concentration (MIC) against particular microorganisms.Unlike the agar diffusion method, which uses solid media, the broth microdilution assay works in liquid culture, which is useful for testing nanoparticles in suspension or liquid formulations. It allows for the comparison of the effectiveness of different concentrations of AgNPs against bacterial strains, helping to optimize their use in applications such as wound healing or water purification.

In this method first prepare nutrient broth then made this sterile by autoclave. Counting the bacteria by hemocytometer. Then prepare two setup like control setup(180microliter media + 20 microliter inoculum) and other one treatment setup in 5 different concentration. Varying amount of media, varying amount of desired drug and 20microliter inoculum put into the 96- well plate. Setting the plate incubation overnight. After the incubation , next day INT dye added and incubated for 30 min.

Role of INT dye in Broth micro dilution assay

In the broth microdilution assay, INT dye (iodonitrotetrazolium chloride) serves as a colorimetric indicator of microbial growth and viability. This tetrazolium salt is initially colorless but is reduced by metabolically active cells to form a red or pink-colored formazan product. When added to the wells after incubation, the presence of a color change indicates microbial growth, while wells that remain

colorless suggest inhibition of growth by the tested antimicrobial agent. The use of INT dye enhances the visual detection of microbial activity, making it easier to determine the minimum inhibitory concentration (MIC) of the compound being tested. This dye is especially useful in assays where turbidity alone may not clearly differentiate between growth and inhibition.

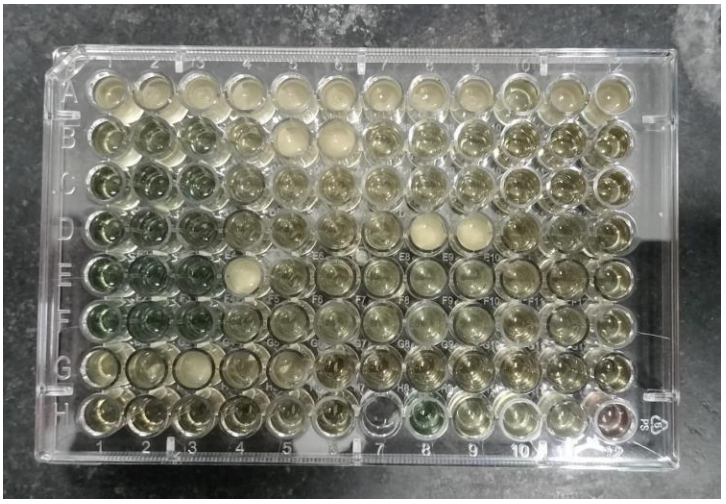


Fig 3.6: 96 well plate use for broth micro dilution assay

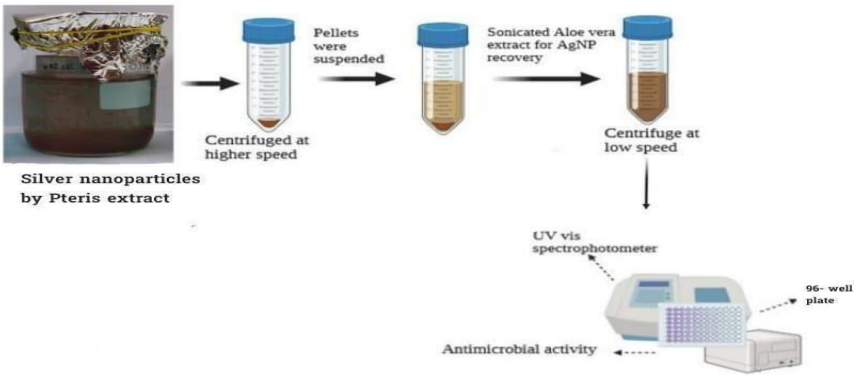


Fig 3.7: Broth micro dilution assay technique

4. RESULT AND DISCUSSION:

❖ Invitro synthesis of silver nanoparticles:

The in vitro synthesis of silver nanoparticles using Pteris plant extract represents an eco-friendly and sustainable approach to nanomaterial production. The Pteris plant, rich in phytochemicals such as flavonoids, phenolics, and terpenoids, acts as a natural reducing and stabilizing agent. When its aqueous extract is mixed with a silver nitrate ($AgNO_3$) solution, the silver ions (Ag^+) are reduced to silver nanoparticles (Ag^0), indicated by a visible color change in the solution. This biosynthesis method eliminates the need for harmful chemicals and high-energy processes typically used in conventional nanoparticle synthesis.

The resulting silver nanoparticles can be characterized using techniques like UV-Vis spectroscopy, FTIR, XRD, and electron microscopy to confirm their size, shape, and crystalline nature. Due to their strong antimicrobial properties, these biogenically synthesized nanoparticles have potential applications in medicine, environmental remediation, and material science.

4A

Fig4A: AgNo3 solution

4B

Fig 4B: Pteris extract

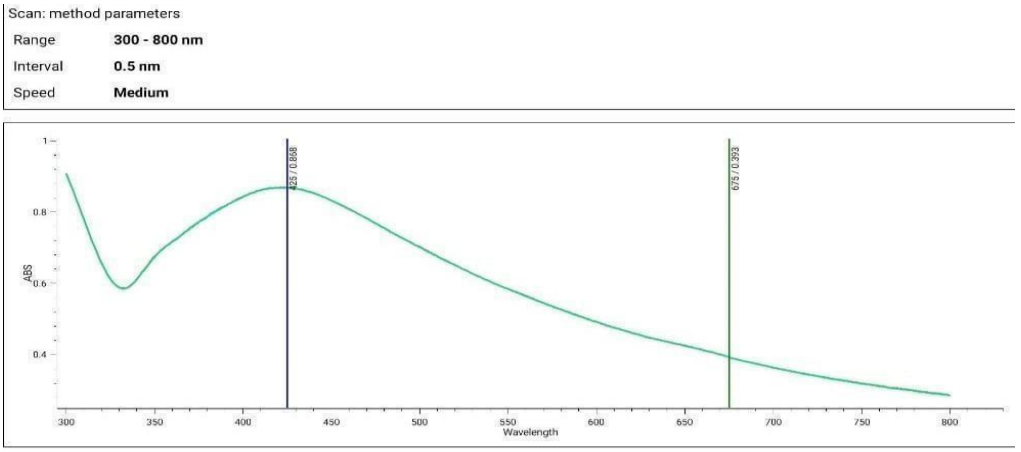
4C

Fig 4C: AgNo3 nanoparticles formation.

- **UV-Visible spectrophotometric analysis:**

The graph represents a UV-Vis absorption spectrum for samples labeled $AgNO_3$ Pteris extract nano 1, 2, and 3, measured across a wavelength range of 300–800 nm with a 0.5 nm interval and medium scan speed. This spectrum is typically used to assess the optical properties of nanoparticles, in this case, silver nanoparticles synthesized using a tea extract (TE). A prominent absorption peak appears at 425 nm, with absorbance values around 0.868–0.869, which is characteristic of the Surface Plasmon Resonance (SPR) of silver nanoparticles, indicating their successful formation. A secondary, weaker absorption feature is observed at 675 nm with absorbance values around 0.393–0.394, likely due to residual organic compounds from the tea extract or slight nanoparticle aggregation.

All three samples exhibit nearly identical absorbance at both wavelengths, suggesting high reproducibility in the synthesis process and uniform nanoparticle characteristics. The strong SPR peak at 425 nm is indicative of spherical AgNPs, typically 20–50 nm in size, while the consistency among samples points to a well-controlled synthesis and stable colloidal dispersion. Overall, the UV-Vis data confirm the successful green synthesis of silver nanoparticles using tea extract, with excellent reproducibility and dispersion observed across all batches.



Sample	ABS(425)	ABS(675)
AgNO3 TE nano 1	0.868	0.393
AgNO3 TE nano 2	0.868	0.393
AgNO3 TE nano 3	0.869	0.394

Fig 4.1: UV – visible spectroscopy analysis of silver nanoparticles between 300 to 800 nm at room temperature

XRD ANALYSIS OF SILVER NANOPARTICLES:

The X-ray diffraction (XRD) pattern presented is typical of silver nanoparticles, indicated by the broad and diffuse nature of the peaks. The graph shows intensity (in counts per second, CPS) versus the 2-theta angle (in degrees), ranging from 10° to 70°. The pattern reveals a high background and broad peaks, which are characteristic of nanocrystalline or poorly crystalline materials.

The broadness of the peaks, especially visible between 30° and 50°, suggests that the silver particles are in the nanometer size range due to the Scherrer broadening effect. However, the exact crystallographic planes (typically (111), (200), (220), and (311) for face-centered cubic silver) are not distinctly resolved in this raw data, possibly due to significant noise or small particle size. A moredetailed analysis including background subtraction and peak fitting would help identify the exact crystallographic nature and estimate particle size. Overall, the pattern supports the presence of silver in nanoparticle form, consistent with a nanostructured or amorphous-like profile.

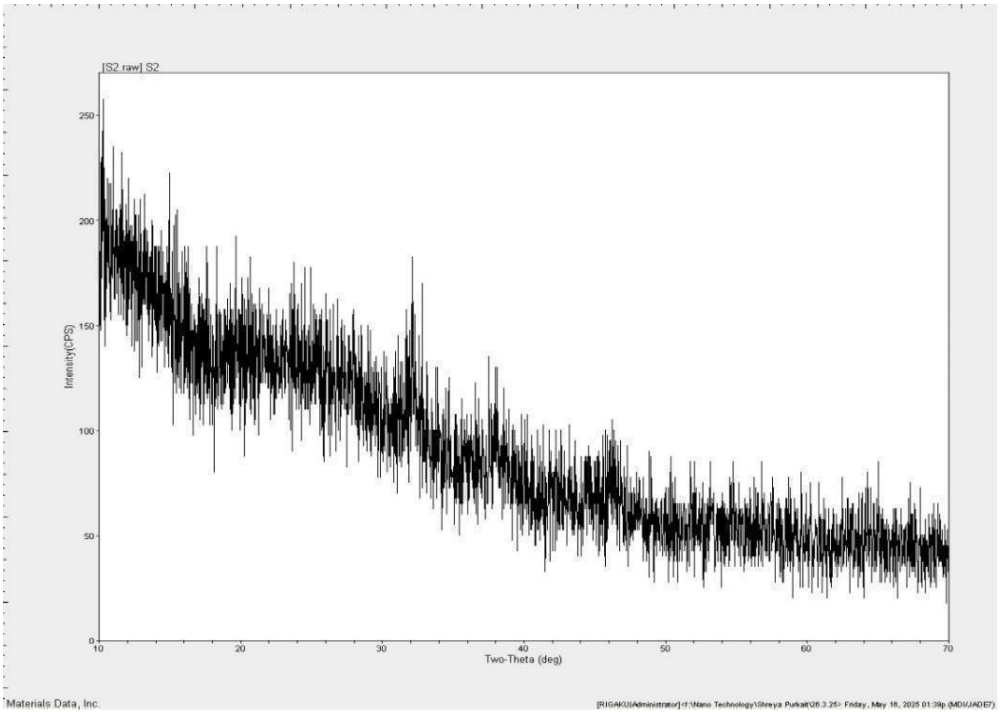


Fig 4.2 : XRD patterns showing peaks corresponding to the diffraction from 111, 200, 220,311 planes of fcc lattice of silver nanoparticles.

- **FTIR ANALYSIS OF SILVER NANOPARTICLES:**

The FTIR (Fourier Transform Infrared) spectrum provided shows the absorbance of silver nanoparticles as a function of wavelength, ranging from approximately 500 nm to 4500 nm (or 2000–500 cm^{-1} in wavenumbers, when converted). The spectrum exhibits several prominent peaks indicating the presence of various functional groups on the surface of the silver nanoparticles, likely due to capping or stabilizing agents used during synthesis.

Notable features include a broad absorbance peak around 3300–3500 nm, which is typically associated with O–H stretching vibrations, suggesting the presence of hydroxyl groups (possibly from alcohols or phenols). Peaks near 2900–3000 nm could correspond to C–H stretching vibrations from aliphatic hydrocarbon chains. The sharp peaks around 1600–1700 nm indicate C=O stretching vibrations, suggesting the presence of carbonyl groups, such as from ketones, aldehydes, or carboxylic acids. Additional smaller peaks in the range of 1000–1500 nm may correspond to C–N or C–O stretching, which are common in amines or ethers.

These functional groups are typically derived from plant extracts, polymers, or surfactants used in green synthesis or chemical reduction methods of silver nanoparticles. Their presence confirms successful surface modification or stabilization, which prevents nanoparticle agglomeration and enhances biocompatibility. Overall, this FTIR spectrum supports the presence of surface-bound organic molecules, confirming the effective functionalization of the silver nanoparticles**.**

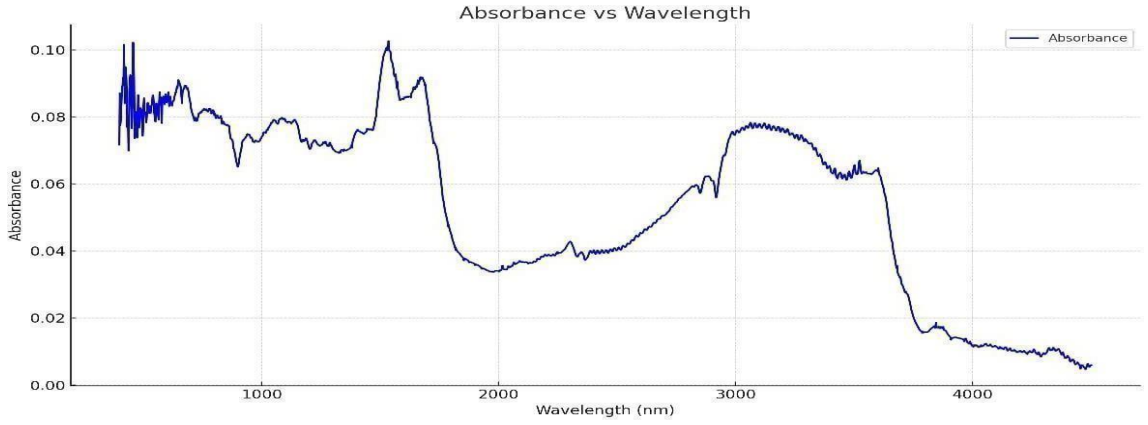


Fig 4.3: FTIR absorption spectra of silver nanoparticles

- **Evaluation of antimicrobial activity of silver nanoparticles:**

Silver nanoparticles (AgNPs) exhibit strong antimicrobial activity against both gram-positive (*Bacillus sp*) and gram-negative (*Escherichia coli*) bacteria due to their unique physicochemical properties. These nanoparticles can attach to bacterial cell membranes, disrupt their integrity, and penetrate inside the cells, leading to damage of DNA, proteins, and other essential cellular components. Gram-negative bacteria, with their thinner peptidoglycan layer and outer membrane, are often more susceptible to AgNPs, although Gram-positive bacteria are also effectively inhibited. The antimicrobial mechanism includes the release of silver ions, generation of reactive oxygen species (ROS), and direct interaction with microbial enzymes and proteins, making AgNPs a promising agent in combating bacterial infections and antibiotic resistance.

SL NO	AgNo3 NPs	*E.coli*	*Bacillus sp.*	Zone of inhibition			
				E.coli Net Zone(cm)	*Bacillus sp.* Net Zone(cm)	Susceptible	Resistance
1	Control	0	0	0	0		R
2	100%	4	4	3.5	3.5	S	
3	50%	2.9	3	2.4	2.5	S	
4	25%	1.2	2.2	0.7	1.7		R

Fig 4.4: Zone of inhibition of *E.coli* plate

Fig 4.5: zone of inhibition of *Bacillus sp.* plate

Table 4.1 Measurement of Zone of Inhibition

Zone of inhibition (mm)= (2× radius) + disc diameter

- Agar diffusion assay:

The agar diffusion assay evaluating the antimicrobial activity of silver nanoparticles (AgNPs) demonstrated significant inhibitory effects against both Gram-positive and Gram-negative bacteria. The results showed that AgNPs exhibited dose-dependent antibacterial activity, with increasing concentrations leading to a marked reduction in bacterial growth.

Gram-negative bacteria such as *Escherichia coli* showed greater susceptibility to AgNPs, indicated by lower minimum inhibitory concentrations (MICs), likely due to their thinner peptidoglycan layer and unique outer membrane structure that facilitates nanoparticle penetration. In the upper result, we see the net zone of *E.coli* is 3.5 cm which is greater than 2.8cm so it is susceptible in 100% concentration of drug. In 50% concentration the net zone is 2.4 which is also susceptible means the microbes can be effectively inhibited by drug . On the other hand in control and 25% concentration, *E.coli* is resistant means the microbes can survive and even grow due to the low concentration of the drug and in control the absent of the drug.

Conversely, Gram-positive bacteria like *Bacillus subtilis* were comparatively less sensitive, potentially due to their thicker cell walls that act as a barrier to nanoparticle entry. In 100% and 50% concentration the net zone of *Bacillus sp.* Is accordingly 3.5 and 2.5 which is susceptible . But in control and 25% , due to the low and absent of drug, net zone is 0 and 1.7 in which the microbes is resistant means it can survive.

Overall, the agar dilution assay confirmed that silver nanoparticles possess broad-spectrum antimicrobial activity, with a slightly higher efficacy against Gram-negative organisms.

- Broth microdilution assay:

The broth microdilution assay revealed distinct susceptibility profiles for Escherichia coli and Staphylococcus aureus. For *E. coli*, the minimum inhibitory concentrations (MICs) indicated moderate to high susceptibility to commonly used antibiotics such as ciprofloxacin and gentamicin, with MIC values falling well within the susceptible range defined by CLSI guidelines. However, resistance was observed for ampicillin and tetracycline, with elevated MICs suggesting reduced efficacy. In contrast, S. aureus demonstrated susceptibility to vancomycin and linezolid, with low MIC values, but showed resistance to penicillin and, in some strains, to methicillin, indicating the presence of methicillin-resistant *S. aureus* (MRSA). These results underscore the importance of tailored antimicrobial therapy based on specific MIC profiles to effectively manage infections caused by these pathogens.

The image shows a 96-well microtiter plate used for a broth microdilution assay, a standard method for determining the minimum inhibitory concentration (MIC) of antimicrobial agents against bacteria. Each well contains a liquid medium, and the variation in color across the wells is indicative of microbial growth and metabolic activity.

In this plate, wells with a greenish or yellow-green hue (mostly on the left side, rows C to F, columns 1–6) suggest active bacterial growth, likely due to a pH indicator that changes color as a result of microbial metabolism. Colorless or pale yellow wells typically indicate no growth, signifying effective inhibition by the tested antimicrobial compound. Wells that are dark red to brown (seen in rows A and B, and scattered elsewhere) may represent intense bacterial activity, contamination, or a highly responsive pH change.

The gradient of colors from green to yellow to red likely corresponds to serial dilutions of the antimicrobial agent across the plate—typically, with decreasing concentration from left to right or top to bottom.

The layout and varied reactions indicate that multiple conditions or bacterial strains might be tested simultaneously. The edge wells (especially the bottom right corner) may serve as controls for sterility, media, or growth. Overall, this plate appears to have generated meaningful data for determining antimicrobial effect.

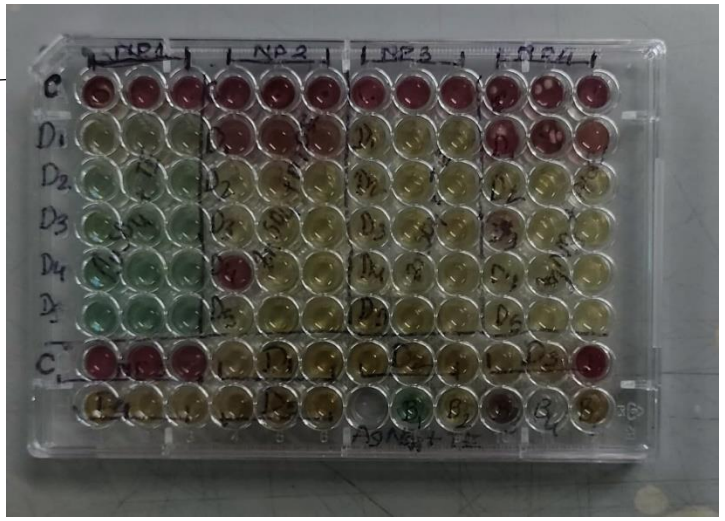


Fig4.6: Antimicrobial activity experiment by Broth dilution assay

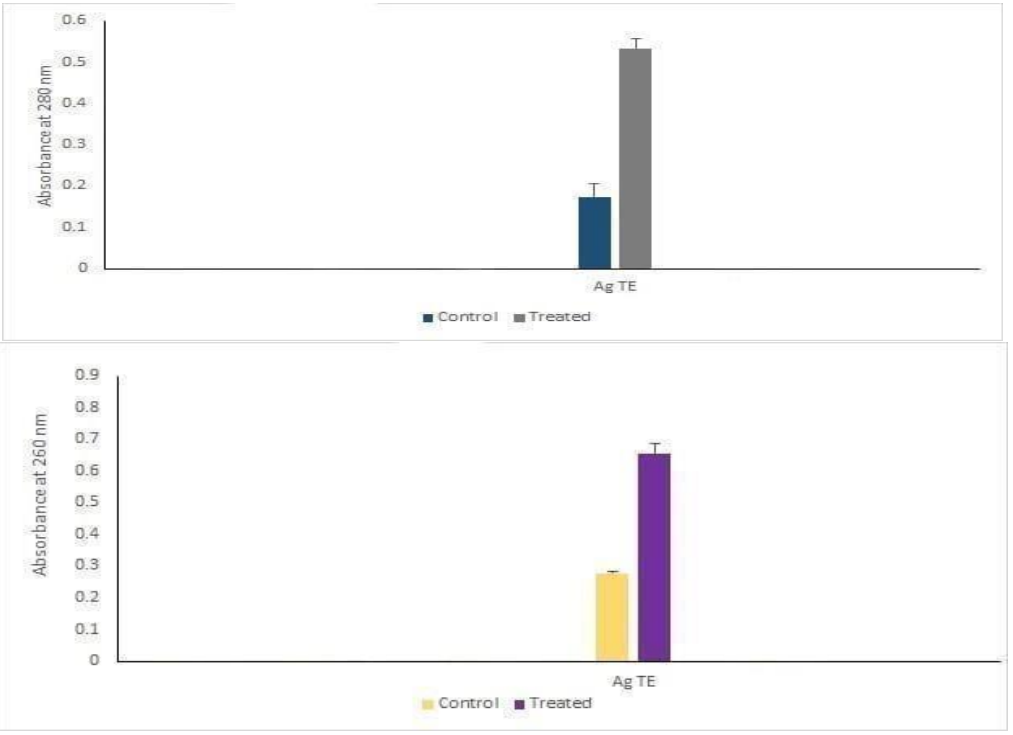


Fig 4.7 : DNA and protein leakage in E. coli cells treated with AgNO3 NPs

This bar graph presents the results of a broth microdilution assay conducted on the measuring absorbance at 260 nm of *E.coli* to assess membrane integrity. The x-axis represents sample conditions, with "Ag TE" likely indicating treatment with a silver-based Pteris extract, while the y-axis shows absorbance values, reflecting nucleic acid leakage—a marker of cell membrane disruption. The yellow bar corresponds to the control group (untreated E. coli), and the purple bar represents the treated group. The treated group shows that the significantly higher absorbance at 260 nm compared to the control. It suggested that the substantial leakage of intracellular contents such as DNA and RNA. This indicates that the Ag TE treatment compromised the bacterial cell membrane, potentially causing cell lysis. The antimicrobial activity of Ag TE may be attributed to the known membrane-disrupting properties of silver.

In contrast, the control group exhibited low absorbance, indicating minimal cellular leakage and intact membranes. Overall, the data support the conclusion that Ag TE exerts have strong antibacterial effects that damaging the cell membrane and inducing nucleic acid leakage of *E.coli.*

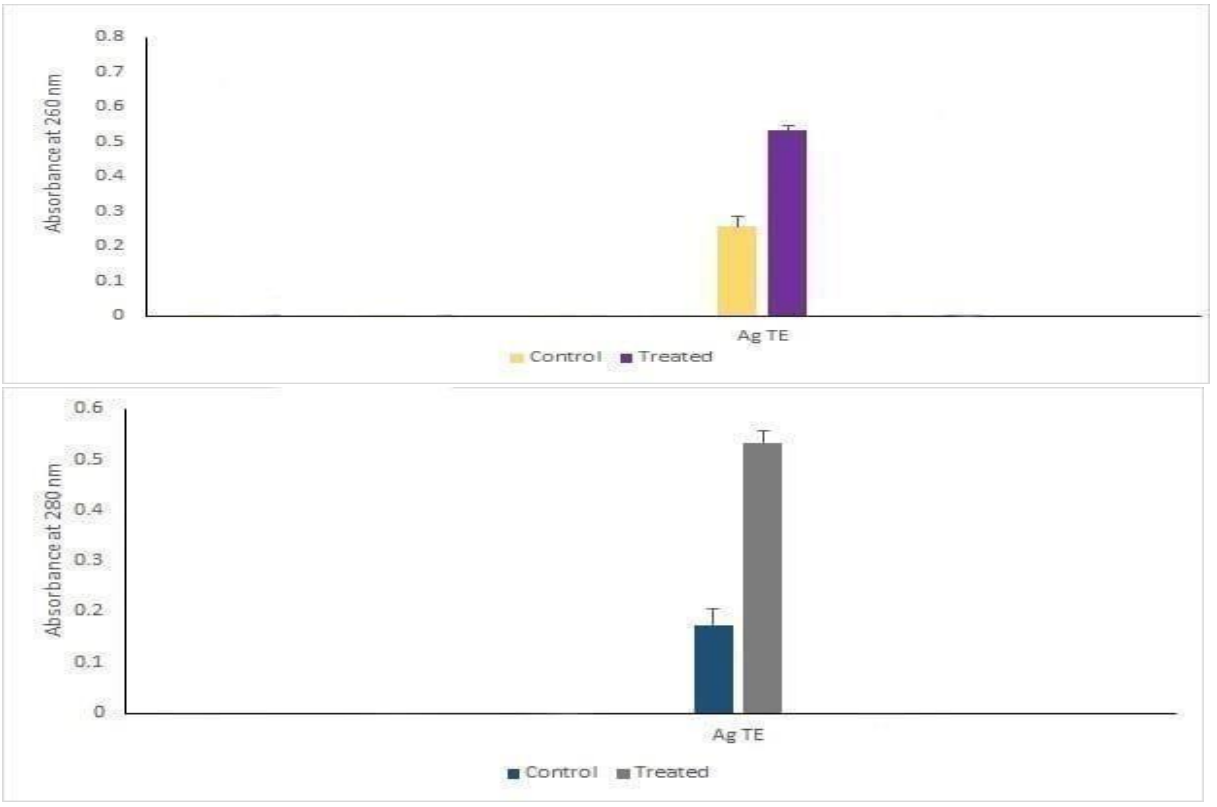


Fig 4.8: DNA and protein leakage in S. aureus cells treated with AgNO3 NPs

The graph illustrates the results of a broth microdilution assay assessing the effect of a treatment on *Staphylococcus sp*. using absorbance measurements at 280 nm as an indicator of protein content or cell density. The x-axis labels a single sample type, "Ag TE" (likely referring to a silver-based Pteris extract), and the y-axis indicates absorbance levels at 280 nm.

Two bars are shown for the "Ag TE" group: one for the Control (untreated) and the other for the Treated sample. The control group has a lower absorbance value (around 0.15), whereas the treated group shows a significantly higher absorbance (approximately 0.55). This increase suggests that the treatment led to a rise in protein content or cellular material in the medium, which might indicate enhanced lysis of bacterial cells, leading to the release of intracellular contents into the broth.

The presence of error bars indicates that the data includes replicates, and the treatment effect is statistically consistent. Overall, this result suggests that the Ag TE treatment has a significant impact on *Staphylococcus sp*., possibly by disrupting the cells and releasing the proteins component into the broth.

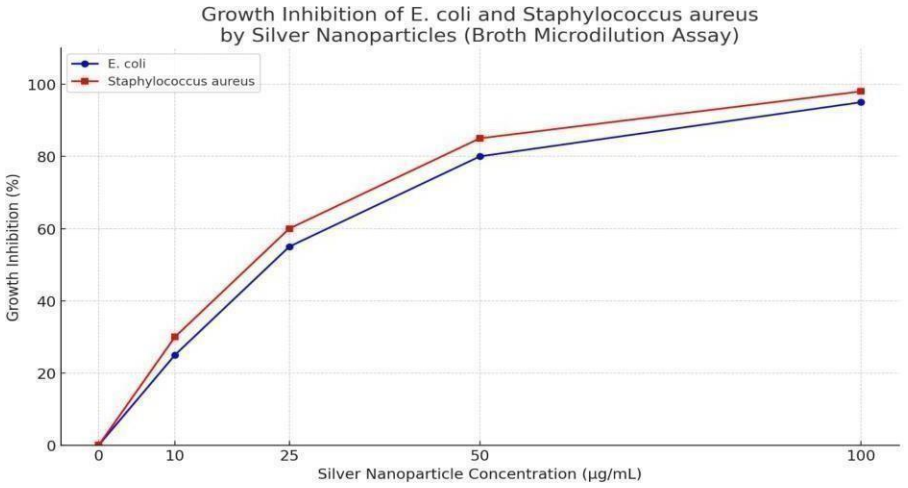


Fig 4.9: Growth inhibition curve of E.coli and Staphylococcus aureus by silver nanoparticles

Here is the graph showing the growth inhibition percentage of *E. coli* and *Staphylococcus aureus* treated with silver nanoparticles using a broth microdilution assay.

DLS analysis of Silver Nanoparticles:

Dynamic Light Scattering (DLS) analysis provides valuable information about the hydrodynamic size and distribution of silver nanoparticles (AgNPs) in a colloidal solution. The DLS result of silver nanoparticles synthesized using Pteris extract typically shows a narrow size distribution, indicating uniformity in particle size. The average hydrodynamic diameter of the AgNPs is often observed to be in the range of 20–100 nm, depending on synthesis conditions such as pH, temperature, and concentration of the extract and silver nitrate.

The polydispersity index (PDI), which indicates the degree of size variation in the sample, is ideally below 0.3, suggesting a relatively monodisperse and stable nanoparticle population. Additionally, the zeta potential value—often found in the range of −20 mV to −40 mV—indicates good colloidal stability due to electrostatic repulsion among particles, preventing aggregation.

In summary, DLS analysis confirms that the silver nanoparticles synthesized via Pteris extract are nanoscale, well-dispersed, and stable, making them suitable for various biological applications, including antimicrobial uses.

5. CONCLUSION:

In conclusion, the green synthesis of silver nanoparticles (AgNPs) using Pteris extract presents an eco-friendly, cost-effective, and sustainable alternative to conventional chemical methods. The phytochemicals present in the Pteris extract act as natural reducing and stabilizing agents, facilitating the formation of stable silver nanoparticles without the use of toxic chemicals. Characterization studies confirm the successful synthesis of AgNPs, typically exhibiting desirable size and morphology for biological applications. Importantly, the biosynthesized AgNPs demonstrate significant antimicrobial activity against a range of pathogenic microorganisms, suggesting their potential use in biomedical applications such as wound dressings, coatings, and disinfectants. This study highlights the promise of plant-mediated nanoparticle synthesis as a green nanotechnology approach, offering a safer and more sustainable path toward the development of novel antimicrobial agents.

6. APPENDIX:

❖ Table 6.1 Nutrient Broth Composition:

Component	Amount (g/L)	Function
Peptone	5.0 g	Source of nitrogen, peptides, and amino acids
Beef extract	3.0 g	Provides vitamins, minerals, and nutrients
Sodium chloride (NaCl)	5.0 g	Maintains osmotic balance
Distilled water	1000 mL	Solvent
pH	~7.0	Adjusted as needed before sterilization

❖ Table 6.2 Standard LB Broth Composition (per liter):

Component	Amount (g/L)	Function
Tryptone (Peptone)	10.0 g	Provides peptides and amino acids (nitrogen source)
Yeast extract	5.0 g	Provides vitamins, minerals, and growth factors
Sodium chloride (NaCl)	10.0 g	Maintains osmotic balance
Distilled water	1000 mL	Solvent
pH	~7.0	Adjusted before autoclaving

❖ Table 6.3 Preparation of 0.01 M Silver Nitrate Solution

Component	Amount
Molarity (M)	0.01 mol/L
Molar mass of $AgNO_3$	169.87 g/mol
Volume of solution	100 mL
$AgNO_3$ required	0.1699 g (≈ 0.17 g)

Solvent	Distilled or deionized water
Final volume	Make up to 100 mL
Storage	Amber bottle, room temperature

7. REFERENCES:

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